

AMENDMENTS TO THE CLAIMS

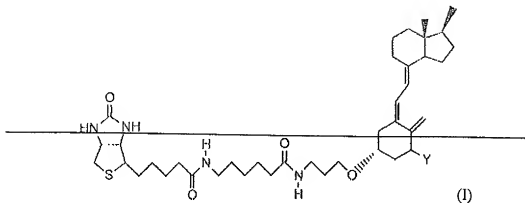
1. - 15. (Canceled)

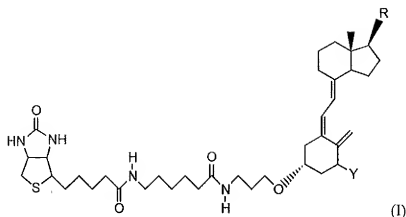
16. (Currently amended) A method of measuring the amount of 1 α ,25-dihydroxy vitamin D in human serum using a competitive protein binding assay, comprising:

i) separating 25-hydroxy vitamin D from the 1 α ,25-dihydroxy vitamin D by binding 1 α ,25-hydroxy vitamin D in a sample of the human serum to a material that specifically binds 1 α ,25-hydroxy vitamin D and eluting 1 α ,25-dihydroxy vitamin D from said material to provide a measurement sample,

ii) measuring the displacement of a vitamin D derivative of formula (I) from an antibody that specifically binds 1 α ,25-dihydroxy vitamin D by adding an amount of the measurement sample to a sample of the antibody having the vitamin D derivative of formula (I) bound thereto.

~~wherein a displacement efficiency of approximately 1 is obtained by using a vitamin D derivative of formula~~





wherein:

R represents a ~~25-hydroxylated~~ 25-hydroxylated side-group of vitamin D₂ or of vitamin D₃, and Y represents hydroxy; and

iii) correlating the measurement of displacement of the vitamin D derivative of formula (I) from said antibody by 1 α ,25 dihydroxy vitamin D present in the measurement sample to the measurement of displacement of the 1 α ,25-dihydroxy vitamin D from the antibody using a known quantity of the vitamin D derivative of formula (I) to determine the amount of 1 α ,25-dihydroxy vitamin D in the sample.

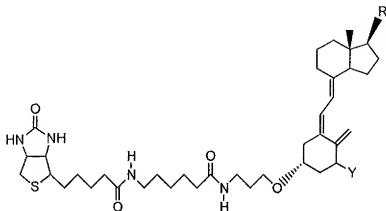
17. - 18. (Canceled)

19. (New) The method of claim 16, wherein said competitive protein binding assay is selected from the group consisting of an enzyme immunoassay, an enzyme-linked immunosorbent assay, a radioimmunoassay, an immunoradiometric assay, a luminescence assay, a fluorescence immunoassay and an immunofluorometric assay.

20. (New) The method of claim 16, wherein the method is a sandwich immunoassay, selected from the group consisting of immunoradiometric assay, IEMA/EIA, immunoluminometric assay and immunofluorometric assay.

21. (New) A kit for determining the concentration of 1 α ,25-dihydroxy vitamin D in a sample of human serum by an immune-based competitive protein binding assay, comprising a standardized

quantity of a solid vitamin D derivative of formula (I) or a standardized solution of a vitamin D derivative of formula (I),



wherein R represents a 25-hydroxylated side-group of vitamin D₂ or of vitamin D₃, and Y represents hydroxy;

a standardized quantity of an antibody that specifically binds 1 α ,25-dihydroxy vitamin D;
and a known quantity of 1 α ,25-dihydroxy vitamin D,

so that the displacement of the vitamin D derivative of formula (I) from said antibody as effected by the 1 α ,25-dihydroxy vitamin D present in the measurement sample can be correlated to the displacement of the vitamin D derivative of formula (I) from said antibody as effected by the addition of a known quantity of the 1 α ,25-dihydroxy vitamin D to determine the amount of 1 α ,25-dihydroxy vitamin D present in human serum.

22. (New) The kit of claim 21, further comprising a material that can bind 1 α ,25-dihydroxy vitamin D for separation of 25-hydroxy vitamin D from 1 α ,25-dihydroxy vitamin D.

23. (New) The kit of claim 21, wherein said competitive protein binding assay is selected from the group consisting of an enzyme immunoassay, an enzyme-linked immunosorbent assay, a radioimmunoassay, an immunoradiometric assay, a luminescence assay, a fluorescence immunoassay and an immunofluorometric assay.

24. (New) The kit of claim 21, wherein said competitive binding assay is a sandwich immunoassay, selected from the group consisting of immunoradiometric assay, IEMA/EIA, immunoluminometric assay and immunofluorometric assay.
25. (New) The kit of claim 21 comprising a solid phase selected from the group consisting of a microtitration plate, another solid carrier, a microparticle, a polymeric material, and a cellulose.
26. (New) The kit of claim 19, in which the solid phase is a microparticle comprising agarose.
27. (New) The kit of claim 19, in which the solid phase is a magnetic microparticle.
28. (New) The kit of claim 22, in which the material that can bind $1\alpha,25$ -dihydroxy vitamin D for separation of 25-hydroxy vitamin D from $1\alpha,25$ -dihydroxy vitamin D is one suitable for packing into a chromatographic column or one that is provided in a chromatographic column.